TECHNICAL MEMORANDUM

PREPARED FOR: StarKist Samoa, Inc.
Chicken of the Sea (COS) Samoa Packing Company, Inc.

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DATE: 31 December 1998

SUBJECT: **Bioassay Testing of Effluent**
November 1998 Sampling

PROJECT: 147323.JC.EM

Purpose

This memorandum presents the results of the bioassay testing of the Joint Cannery Outfall effluent sample that was collected in November 1998. This is the twelfth required semi-annual test. Separate technical memoranda are being prepared to describe the results of concurrent effluent chemistry testing.

Study Objectives

Section D.1 of the StarKist Samoa and COS Samoa Packing NPDES permits requires that semi-annual definitive acute bioassays (96-hour static bioassays) be conducted on the cannery effluent. The purpose of these bioassays is to determine whether, and at what effluent concentration, acute toxicity may be detected for the effluent.

U.S. EPA has conducted a number of reviews of the effluent sampling, analysis, and bioassay tests. All comments from U.S. EPA have been incorporated into either the Standard Operating Procedures or have been incorporated into the procedures by the laboratory doing the test, Advanced Biological Testing, Inc., as documented in previous reports.

The bioassays were originally specified to be conducted using the white shrimp, *Penaeus vannami* (postlarvae). In the event *Penaeus vannami* is not available at the time of the tests, a substitute species, *Mysidopsis bahia*, has been approved by U.S. EPA (CH2M HILL, 26 January 1995). For the November 1998 sampling, *Penaeus vannami* was not available and *Mysidopsis bahia* was used.

The acute bioassay effluent sampling must be concurrent with effluent sampling for

chemical analysis. Effluent samples are to be collected as 24-hour composite samples. The effluent acute bioassay was conducted using a combined composite effluent sample made up from the composite effluent samples from the StarKist Samoa and COS Samoa Packing facilities, as approved by EPA. This combined effluent bioassay is representative of the wastewater discharged from the joint cannery outfall to Pago Pago Harbor.

Effluent Sampling Methods

Between 1200 on 19 November 1998 and 0900 on 20 November 1998, 24-hour, flow-weighted, composite samples of final effluent were collected from both the StarKist Samoa and COS Samoa Packing effluent discharges. Samples were collected from the established effluent sampling sites following the routine composite sample collection schedule for the plants. Detailed sampling procedures are described in the technical memorandum presenting the March 1995 effluent bioassay sampling.

A total of eight grab samples were collected into pre-cleaned 1-gallon plastic cubitainers at each plant. Samples were collected at approximately three-hour intervals over a 24 hour period. The samples were stored on ice until the completion of the 24-hour sampling period. After all samples were collected a flow-proportioned composite sample was prepared. The grab sample collection times and the relative effluent volumes calculated from plant flow records are summarized in Table 1. The relative effluent volumes were used to prepare the final composite sample, which was used to fill the sample container shipped to the laboratory for testing.

A 5-gallon cubitainer containing the composite sample was packed on ice in an ice chest for shipment to the laboratory. A chain-of-custody form for the sample was completed and then sealed into a zip-lock bag and taped inside the lid of the ice chest. The sample was shipped via DHL on flights from Pago Pago to Honolulu and then to San Francisco. Samples were received by the testing laboratory on 23 November 1998. The chain-of-custody form is provided in Attachment I.

Bioassay Testing Procedures

The bioassay tests were conducted by Advanced Biological Testing Inc., Rohnert Park, California. The testing procedures and results of the bioassay tests are provided in "*Results of a Bioassay Conducted on an Effluent Sample from the Joint Cannery Outfall in American Samoa Using Mysidopsis bahia*" dated 17 December 1998 included as Attachment II. This report summarizes the 96-hour acute bioassay test conducted with reference to U.S. EPA document *EPA/600/4-90/027F,* August 1993, as the source of methods for conducting the test.

The bioassay test was conducted considering and including U.S. EPA's comments on previous bioassay tests, as documented in previous reports. A brine control was run and a comparison was made with the dilution water "laboratory control". The test organisms were required to be 1 to 5 days old, with a 24-hour range in age, and the test temperature was to be held at 20 ±1°C or 25 ±1°C. For this bioassay, three day old *Mysidopsis bahia* were used since penaeids were not available. Mysids were tested at 25 ±2°C. Because of the demonstrated potential for a lethal immediate dissolved oxygen demand (IDOD), discussed and documented in previous technical memoranda describing the first two bioassay tests, each bioassay test chamber was continuously aerated during the bioassay tests to maintain adequate levels of dissolved oxygen (DO). Bioassay tests were carried out for effluent concentrations of 50, 25, 12.5, 6.25, and 3.1% as vol:vol dilutions in seawater. Water quality was monitored daily and parameters measured included DO, pH, salinity, temperature, and ammonia. Additionally, a reference toxicant of sodium dodecyl sulfonate (SDS) was made up of a 2-gram per liter stock solution in distilled water and tested at concentrations of 25, 12.5, 6.25, 3.1, and 1.9 mg/L in 31 ppt seawater for a 96-hour test.

Results

The results of the bioassay tests are summarized as follows:

> ***Mysidopsis bahia* Effluent Bioassay.** All results from the bioassay tests are included in Attachment II. The results of the mysid bioassay tests indicate the LC_{50} for the effluent tested was 15 percent. The No Observable Effects Concentration (NOEC) for the 96-hour bioassay was 6.25 percent and the Least Observable Effects Concentration (LOEC) was 12.5 percent. The calculated value of toxicity units (TU) was 16.
>
> ***Mysidopsis bahia* Reference Toxicant Bioassay.** The reference toxicant had a LC_{50} of 15.2 mg/l. The laboratory mean was 14.53 ± 5.06 mg/l with the data falling within one standard deviation of the laboratory mean, indicating normal sensitivity.

Discussion

Table 2 summarizes the results of the effluent bioassay tests for the samples collected in the November 1998 sampling compared to the previous bioassay tests. The LC_{50}, NOEC and LOEC are within the range obtained from previous reports where *Mysidopsis bahia* was used in place of *Penaeus vannami*.

Conclusions

The bioassay tests for the Joint Cannery Outfall effluent for November 1998 do not

indicate effluent toxicity levels to be of concern. As discussed in the previous bioassay test reports on the effluent, the time scale of the mixing of the effluent with the receiving water is on the order of minutes to seconds to achieve dilutions that will eliminate possible toxic effects as reflected by the bioassay results. For example, an NOEC of 6.25% which was observed in November 1998, corresponds to a dilution of 16:1 which is achieved within a time frame of seconds and within a few meters of the discharge point. The discharge is located in about 180 feet of water and the effluent toxicity tests indicate that the discharge is diluted to non-toxic levels immediately after discharge and well within the initial dilution plume.

Table 1
StarKist Samoa and COS Samoa Packing 24-hour Composite Effluent Sample for Bioassay Testing
19 – 20 November 1998

Grab Sample Number	COS Samoa Packing		StarKist Samoa		COS Samoa Packing Percent of Total Flow	StarKist Samoa Percent of Total Flow
	Sampling Date and Time	Effluent Flow Rate (mgd)	Sampling Date and Time	Effluent Flow Rate (mgd)		
	11/19/98		11/19/98			
1	1200	0.88	1200	1.82	4.6	9.5
2	1500	0.88	1500	1.65	4.6	8.6
3	1800	0.88	1800	1.29	4.6	6.7
4	2100	0.80	2100	1.47	4.2	7.7
5	2400	0.80	2400	1.46	4.2	7.6
	11/20/98		11/20/98			
6	0300	0.80	0300	1.50	4.2	7.8
7	0600	0.80	0600	1.61	4.2	8.4
8	0900	0.90	0900	1.59	4.7	8.3
Total		6.74		12.39	35.3	64.6
Mean		0.84		1.55		

Table 2
StarKist Samoa and COS Samoa Packing
Combined Effluent Bioassay Results

Date	*Species*	Parameters		
		LC 50	NOEC	LOEC
2/93	*Penaeus vannami*	4.8%[1]	3.1%	6.25%
10/93	*Penaeus vannami*	15.67%	3.1%	6.25%
2/94	*Penaeus vannami*	15.76%	<1.6%	1.6%
10/94	***Mysidopsis bahia***[2]	**31.2%**	**25%**	**50%**
3/95	*Penaeus vannami*	14.8%	6.25%	12.5%
3/95	***Mysidopsis bahia***[3]	**10.8%**	**6.25%**	**12.5%**
2/96	*Penaeus vannami*	>50%	>50%	>50%
2/96	***Mysidopsis bahia***[3]	**28.36%**	**12.5%**	**25%**
3/96	*Penaeus vannami*	44.4%	25%	50%
11/96	*Penaeus vannami*	7.11%	3.1%	6.25%
03/97	*Penaeus vannami*	39.36%	12.5%	25%
09/97	*Penaeus vannami*[4]	12.3%	6.25%	12.5%
06/98	***Mysidopsis bahia***[2]	**17.2%**	**6.25%**	**12.5%**
11/98	***Mysidopsis bahia***[2]	**15%**	**6.25%**	**12.5%**

[1]The February 1993 samples were not aerated until after the first day of the test. For subsequent tests the samples were aerated for the entire duration of the tests.
[2]*Mysidopsis bahia* substitutes as *Penaeus vannami* not available, as directed by U. S. EPA.
[3]*Mysidopsis bahia* used in addition to *Penaeus vannami* as described in text. Only one species is required by the permit conditions.
[4]Stage 1 (3 mm) *Penaeus vannami* were used for testing as older Stage 7 and 8 (8-10 mm) *Penaeus vannami* were not available.

ATTACHMENT I

CHAIN-OF-CUSTODY FORM

JOINT CANNERY OUTFALL EFFLUENT SAMPLE
19 – 20 November 1998

ATTACHMENT II

LABORATORY REPORT
Advanced Biological Testing
96-hour Acute Bioassay

JOINT CANNERY OUTFALL EFFLUENT SAMPLE
19 – 20 November 1998